1991 Report of the DNA Commission of the Society for Forensic Haemogenetics conerning the use of DNA polymorphisms

"The time has come" the Walrus said "to speak of many things": Lewis Carroll.

1. Introduction

In 1989 the ISFH published its first recommendations concerning the application of DNA investigations to forensic science (Forensic Sci Int, 1989, 43: 109-11; Vox Sang, 1989, 57: 276-277; Biotech-Forum 1989 6: 111-112). It was obvious, even at the time of publishing, that these guidelines would need revision and updating because of the rapidly changing situation with regards to DNA technology. Since then the DNA commission of the ISFH has met twice with the aim of improving the recommendations so that they encompass the new developments in this field. This report is concerned primarily with the detection of DNA polymorphisms by restriction fragment length polymorphism (RFLP) analysis. It also contains general recommendations applicable to all DNA polymorphism analysis.

2. Definitions of genetic systems and documentation

A genetic locus is defined by a segment of unique DNA sequence that occupies a specific position on a chromosome. Genetic polymorphism at the DNA level can generally be divided into two categories:

a)   polymorphism in sequence resulting from nucleotide base substitutions, and

b)   polymorphism in sequence resulting from insertions or deletions of a nucleotide or nucleotides.

Among the most informative DNA polymorphisms for identification purposes are insertion/deletion polymorphisms containing variable numbers of tandem repeat (VNTR) sequences. These are conveniently detected by restriction fragment length polymorphism (RFLP) analysis.

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2.1 Definition of systems and alleles:

2.1.1

a) A DNA typing system is defined by the designation of a
    genetic locus and the information needed to detect alle-
    lic variation at that locus. RFLP systems are defined by
    the probe and restriction enzymes used for the typing.
    Systems based on gene amplification by the polymerase
    chain reaction (PCR) are defined by the sequences of the
    PCR primers and the method used for detecting sequence
    polymorphism.

    DNA polymorphism detection systems can be divided opera-
    tionally into two groups: single-locus systems (SLS) and
    multi-locus systems (MLS). With the latter, polymorphisms
    at multiple loci are detected simultaneously.

b) At VNTR loci "alleles" are defined by DNA fragments of
    discrete length, which are inherited in agreement with a
    formal genetic model. They are detected by means of con-
    ventional Southern blot analysis or comparable methods.
    They are usually represented by one or two restriction
    fragments of a given size generated by the use of one
    enzyme (or two enzymes in double digestions) and detected
    with one probe. For PCR poducts the fragments can be
    stained directly.

c) VNTR "allele" designation should be preferentially in
    kilobase size, but other methods can be applied if proven
    to be more appropriate (e.g. Rf, molecular weight or al-
    lele number).

2.1.2 Multi-locus systems:

Mutli-locus systems use probes that recognize genomic sequen-
ces which are normally distributed throughout the entire geno-
me. Multi-locus typing gives a pattern of bands, the so-called

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"DNA-fingerprint". Although the pattern of bands (restriction
fragments) is genetically determined, it is not possible to
specify the genetic locus from which each band originates.
Accordingly, the bands in a multi-locus pattern cannot be de-
fined explicitly by a formal genetic model.

2.2 Typing methodology:

It is generally recommended that any method used should be
based on an established protocol.

2.2.1 General and developmental requirements:

a)   Collaboration and exchange of data should be encouraged
      to establish the usefulness of a system and comparability
      of data.

b)   DNA polymorphisms should be defined by family and popula-
      tion studies. At least 500 meioses and an adequate popu-
      lation sample should have been tested and published, be-
      fore a polymorphism can be introduced into paternity te-
      sting.

c)   The chromosomal localisation and linkage data to other
      polymorphisms used in paternity testing should be availa-
      ble. This information should be documented in the publi-
      cations of the International Human Gene Mapping Workshop.

d)   The description must included: DNA sequence data defining
      the locus (if available), proof of Mendelian inheritance,
      "allele" or haplotype frequencies, frequencies of muta-
      tions and/or recombinations and a check using a suitable
      statistical procedure that the population is not out of
      genetic equilibrium. For RFLP systems, the description
      must include: information on the probe and restriction
      enzyme and information on the size of constant and varia-
      ble fragments.

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2.2.2 Requirements for RFLP methodology and standardization:

a)   Size markers with discrete fragments of known size should
      span and flank the entire range of the DNA system being
      tested. Bands which do not conform to this rule should be
      designated appropriately (see 3.2).

b)   A human control DNA of known allele composition should be
      included on each gel. Commercially available cell lines
      (eg K 562) or other control human DNA are considered to
      be suitable.

c)   Intactness of the individual genomic DNA before restric-
      tion enzyme digestion and complete digestion of the DNA
      should be assured by appropriate control experiments. It
      is recognized that there are situations where this may
      not always be possible (e.g. stain work). Under these
      circumstances analysis can still be carried out but the
      results should be interpreted with caution.

3. Establishment of a population data base

3.1 Construction of data bases:

Each laboratory should construct its own data base for appro-
priate local populations. Such data bases should be composed
of not less than 100 individuals.
The population sample should be representative of the relevant
local population(s). Data bases for different local popula-
tions should not be merged until it can be demonstrated that
it is statistically acceptable to do so. Raw data on RFLP
fragment sizes should designate band sizes to at least 10 bp
resolution.
No particular method of fragment size measurement is recommen-
ded. However, for RFLPs automatic methods would be preferred
to manual methods due to their accuracy and reproduceability
but for systems with discrete alleles manual methods can be

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more convenient.

3.2 Fragment (band) size determination:

A good size-marker with an adequate number of fragments is
essential and should be placed at regular intervals to correct
for possible inhomogenetics across the gel. Such a ladder
should span and flank all the fragments to be measured. The
number of bands and the spacing of bands cannot be precisely
stated and must be left to the discretion of the operator de-
pending on the system in question. If a fragment lies outside
the standard ladder this cannot be assigned an accurate size
but simply should be coded as lying above or below a particu-
lar fragment size.

4. Recommendations for paternity testing

4.1 Single-locus system:

4.1.1 Mutation:

a)   The mutation rate for single-locus systems should be
     known.
b)   Systems with high mutation rates should not be used rou-
     tinely and require special considerations:

4.1.2 Matching criteria:

A match is considered to occur when genetic types cannot be
distinguished.

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a)   Comparison of specimens in paternity cases can be made by
     (purely) visual comparison (side-to-side) or co-electro-
     phoresis in the same gel. If the comparison is made from
     different gels a numerical evaluation is essential. A
     mathematical matching rule can be applied to confirm or
     refute a visual match.

b)   The initial visual comparison of bands may be confirmed
     by numerical methods such as Bayes, sliding or fixed
     windows as long as the prerequisites and limitations of
     each method are taken into account.

c)   By definition 2 bands to be compared can be called a
     match if they fall within the limits of the match-window.

d)   The significance of an inconclusive or borderline deter-
     mination may be estimated by the Bayesian approach using
     standard error and standard deviation of the bands in
     combination with rehybridisation using other probes.

4.1.3. Fragment frequency:

a)   A discrete allele system can be unambiguously resolved by
     comparison with suitable ladders of known fragment
     lengths. However suitable biostatistical methods can be
     used to define and check frequencies.

b)   If the fragment distribution is quasi-continuous the fre-
     quency of a single fragment can be estimated according to
     the predefined criteria for matching, but will vary de-
     pending on the method used (i.e. floating or fixed bins).

c)   Under normal circumstances it is essential to estimate
     the frequency of a given fragment size. This requires the
     application of predefined criteria which must be at least
     equal to or greater than the criteria for a match (e.g.
     if matching window = ± 3 x sigma, the corresponding bin
     for frequency estimation must be at least the same or
     greater).

d)   For small data bases with less than 200 individuals con-
     sideration should be given to the measurement errors of

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the frequency estimate.

e) Care must be taken that the population is truly represen-
tative to eliminate sampling error.

f) Bayesian approaches based only on observed fragment sizes
may also be used.

g) It would be preferable to give the correct result concer-
ning the fragment frequency. As this is not possible the
best alternative is to give a conservative estimate. In
the sliding window approach and its variations, the cen-
tre of the window is the point corresponding to the actu-
al measured fragment size. In the fixed bin approach the
actual measured size of the fragment falls somewhere wit-
hin the bin. Conservatism is achieved by moving into ad-
jacent bins if these provide a higher frequency or crea-
ting larger bins. Binning is based on rungs of molecular
weight ladders, or on natural valleys in a fragment dis-
tribution curve.

h) Phenotype frequencies from several systems can be combi-
ned by multiplication unless it has been proved by appro-
priate statistical testing that there is disequilibrium
between systems. At present there is no evidence of dis-
equilibrium between the single-locus systems in common
use.

4.1.4 Compliance to Hardy-Weinbery equilibrium expectation:

a) Population data bases should be checked using suitable
statistical procedures for deviations from the norm. Ho-
wever, potential artefacts exist which can influence the
results of such tests so that apparent significant devia-
tions may also be due to technical problems and should be
investigated further.

b) Estimation of profile (band) frequencies should be tested
for dependence. If non-independence is demonstrated then
frequency estimates incorporating 2 or more probes cannot
be calculated by multiplication of genotype frequencies.

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Either haplotype frequencies should be quoted or a suit-
able statistical analysis used in which it has been de-
monstrated that it is not necessary to make the assump-
tion of independence.

c)   Obvious artefacts must be explained and must be adequete-
ly addressed and taken into account for the interpreta-
tion of results.

4.2 Multi-locus systems:

a)   Mutation rates must be known, but it should be recognized
that rates of 10^{-2} and higher can occur.

b)   Band sizes and/or band patterns should be scored in an
objective manner.

c)   All questions of independence, allelism and linkage dis-
equilibrium need to be addressed and used in a conserva-
tive way if included in biostatistical calculations.

d)   Calculated probabilities on the statistical basis for
paternity are at present still under discussion. Probabi-
lities must be based on full genetic and biostatistical
analysis as outlined in (b) otherwise only a verbal opi-
nion on exclusion or non-exclusion should be given.

4.3 Conclusions:

Paternity testing with conventional techniques is a well
established procedure for producing evidence in court cases,
and can continue to be used either alone or in combination
with DNA polymorphisms. Providing that a DNA system has been
suitably and adequately scrutinised there is no reason why DNA
should not be used alone.

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5. Concerns for identity testing

5.1 Specific requirements for the application in criminal investigations:

In this section some specific requirements are listed with
regard to the analysis of stains; however, it is stressed that
many (but not all) of the requirements discussed previously
are also relevant under this heading. The sections referring
to family studies and to mutation rates are not applicable
except in those cases where identity testing entails testing
of family members such as in missing person cases.

5.2 Somatic stability:

The application of DNA analysis in criminal investigations is
mainly concerned with the comparison of genetic types obtained
from a reference blood sample with those obtained from an evi-
dentiary body fluid or stain. The stain may be a deposit of
blood, semen, vaginal fluid, saliva or even a smear of tissue.
Also the analysis of hairs, in particular hair roots, may be
undertaken. The system used should therefore be shown to be
somatically stable: that is, tissue specific modifications to
DNA (such as methylation) must be shown not to affect genetic
typing determinations.

5.3 Band matching and statistical interpretation:

5.3.1 Genetic typing systems yielding discrete genetic types:

Typing results should be interpreted according to standards
established for blood group and protein genetic markers.

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5.3.2 RFLP typing systems:

In addition to the factors mentioned under paternity testing
it is recognized that the reproducibility of any method may
lead to the imprecise alignment of bands which are neverthe-
less considered to match (for definition see 4.1.2). Further-
more in stain analysis interference from substrates or degra-
dation of the DNA can cause minor variations in band position
again leading to some distortion. Such positional variations
must be shown to be within the expected experimental variation
and wherever possible the statistical assessment should be
correspondingly adjusted. A record of the analysis, the asso-
ciated results and the method of the statistical evaluation
should be readily available for examination by a second inde-
pendant analyst.

5.4 Analysis of semen-contaminated vaginal swabs:

When differential extraction of vaginal swabs is carried out
the supernatant generally containing mainly female DNA may
also be tested, as it can provide a useful internal control.

6. Quality assurance

6.1 Intra-lab quality assurance:

Quality assurance for the individual laboratory is essentially
covered under points 2.2, 3 and 4 of this report. For RFLP
typing systems intra-assay measurement precision (i.e. inter-
and intragel) within one laboratory should be evaluated and
used as the basis of any statistical calculations, in determi-
nation of match window and allelic frequencies.

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6.2 Minimum requirements for inter-lab comparisons:

If it is required to pool or compare data from two separate laboratories, the exchange of a relatively small number (e.g. 20) of samples between labs allows some assessment of the ability to reproduce typing results. For RFLP systems samples spanning the full range of allele fragments are required in order to obtain information as to whether or not results correlate between two laboratories. An adequate number of samples needs to be exchanged between laboratories if databases are exchanges and it is necessary to determine by experiment how much variation exists between the laboratories over the whole range of fragment sizes.

6.3 Blind trials:

Laboratories should participate in appropriate inter-laboratory trials and aim to achieve consensus results. Each laboratory should operate in its own sphere using its own database so long as the required standards have been obtained but to compare results with other laboratories the recommended criteria should have been met.

The Executive Committee of the International Society for Forensic Haemogenetics (B. Brinkmann, R. Bütler, P. Lincoln, W.R. Mayr, U. Rossi) and coopted external experts (W. Bär, M. Baur, B. Budowle, R. Fimmers, P. Gill, K. Hummel, J. Morris, S. Rand, Ch. Rittner, G. Sensabaugh).